

Serum Interleukin 8 Level as a Diagnostic Marker in Late Neonatal Sepsis

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Abstract

Objective: Late-onset sepsis is responsible for high morbidity and mortality in newborn infants in the world and in particular in developing countries. In this study, we evaluated whether clinical characteristics, laboratory parameters and measurements of serum interleukin-8 (IL-8) are able to discriminate between late neonatal sepsis and normal baby.

Methods: This was a prospective (case-control) study conducted between March 2007 and April 2008, at the neonatal intensive care unit, Ghaem Hospital, Mashhad, Iran. The study comprised 93 neonates ≥ 72 hours of life. The infants were categorized in two groups based on the clinical presentation, and biochemical markers including complete blood count, C-reactive protein (CRP) and blood culture: 1) Control group including 42 infants with routine screening and 2) Case group consisting of 38 infants with definitive infection (positive blood and/or cerebrospinal fluid culture) or clinical sepsis (clinical and laboratory signs of infection without positive blood or CSF culture). Receiver-operating characteristic curves were used for the determination of thresholds for the infection group versus healthy neonate group.

Findings: Eighty infants were enrolled in this study. IL-8 and CRP decreased in order of definitive infection, clinical sepsis and healthy subjects respectively ($P < 0.001$). Sensitivity, specificity, positive predictive value, negative predictive value for serum levels were 0.95, 0.1, 0.97, 0.1 for IL-8 and 0.83, 0.86, 0.83, 0.69 for CRP respectively (cut-off point for IL-8 > 60 pg/ml and for CRP > 6 mg/dl).

Conclusion: IL-8 may be a valid and early predictive marker of neonatal infection. Also, IL-8 is associated with severity of infection.

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Key Words: Interleukin-8; C-Reactive Protein; Sepsis; Newborn; Laboratory Marker

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Introduction

Neonatal sepsis is a devastating disease with an incidence of 1 to 10 per 1000 live births and a mortality rate of 15% to 50%^[1]. Bacterial infection contributes significantly to morbidity and mortality in newborn infants^[2,3,4]. The term of late-onset infection refers to the age at onset of infection after third day of life.

Improvement outcome and successful treatment depends on early initiation of appropriate antibiotic therapy, but early diagnosis of neonatal bacterial infections is difficult, because clinical signs are non-specific, variable and may initially be subtle. Isolation of micro organism from blood, cerebrospinal fluid (CSF) and urine is the gold standard method to diagnose a neonatal infection, but microbiological culture is not available until at least 2-5 days. It is therefore recommended for all neonates who develop signs of sepsis to start empirical antimicrobial therapy. Thus, even infants with no sepsis usually receive at least 3-5 days of antibiotic therapy. Therefore, markers are needed to reliably identify truly and early infected neonates^[5]. In recent years, hematological and biochemical markers such as: immature/total neutrophil ratio^[6], platelet count^[7], C-reactive protein (CRP)^[8] and various cytokines^[9,10], have all been suggested as being useful indicators for early identification of septic infants^[11,12]. IL-8, a pro-inflammatory cytokine predominantly produced by monocytes, macrophages, and endothelial cells, rises early in the course of neonatal bacterial infections. Because of the high prevalence of neonatal sepsis in Iran we aimed to compare IL-8 with other laboratory tests including white blood cells (WBC), platelets (PLT) and CRP in the early diagnosis of late neonatal sepsis.

Subjects and Methods

This was a prospective (case-control) study conducted between March 2007 and April 2008, at the neonatal intensive care unit (NICU) and emergency department in Ghaem Hospital,

Mashhad, Iran. Ninety three neonates were eligible for the study. Parental informed consent was obtained for every patient and normal newborn before admission to the study. This study was done with approval of the Ethics Committee of Mashhad University of Medical Sciences.

The neonates were categorized on the basis of their clinical presentation, complete blood count (CBC), CRP and blood culture in: 1) Control group and 2) Cases divided into subgroups of definitive infection and clinical sepsis (Table 1).

Inclusion criteria were positive clinical signs of sepsis and positive blood or cerebrospinal fluid culture as a definitive infection (n=18). Among them, 15 neonates had positive blood culture and 3 cases positive cerebrospinal fluid culture. The patients in subgroup clinical sepsis had clinical and laboratory evidence of infection [CRP>6 mg/dl, platelet count <150000/cmm, WBC>12000/cmm and/or absolute neutrophil count (ANC)>6400/cmm], but negative blood or CSF cultures (n=20). Clinical signs of sepsis were defined as the presence of two or more of the following clinical signs: respiratory compromise (tachypnea, grunting, intercostal retractions, apnea and need to ventilation), gastrointestinal compromise (feeding intolerance and abdominal distension), neurological changes (seizure and irritability), cardiovascular compromise (hypotension and cyanosis), general signs (fever, lethargy).

Exclusion criteria were congenital malformations, congenital infections associated with the TORCH complex, and refusal of parents.

The symptoms were recorded by the resident of pediatrics or neonatologist in the neonatal or emergency unit. Thirteen infants were excluded for the reasons related to problems with specimens, including insufficient blood sample, specimens becoming mislaid or insufficient data for analysis and parental refusal.

Also forty-two healthy infants were recruited as a control group (non-infected newborn was defined as having no clinical or laboratory signs of sepsis, which refer to clinic for screening tests or physiologic hyperbilirubinemia).

Blood culture, cerebrospinal fluid culture, urine culture, CBC with differential, platelet count, blood sugar, urea, creatinin, sodium,

potassium, calcium and serum interleukin-8 (IL-8) were determined at the request of the clinicians at initial evaluation. CRP concentration during 6 hours after initial symptoms also was determined.

From each neonate with suspected infection, blood sample of 1 to 2cc was taken by venipuncture for IL-8 determinations and other laboratory tests. A similar blood sample was also obtained from 42 healthy neonates concurrently for serum bilirubin measurement or other routine tests. Plasma was separated by centrifugation and then stored in aliquots at -70°C until analysis. IL-8 enzyme-linked immunosorbent assay kit (Bender Med system GmbH) was used to determine the serum level of IL-8 (normal value for IL-8 36pg/ml). All samples ran in duplicate. Plasma CRP concentrations were measured immunoturbidimetrically. Levels greater than 6 mg/dl were defined as abnormal.

Data analysis: Descriptive and analyzing tests (Mann-Whitney rank-sum test, Student t-test, chi-square test, Pearson correlation and Spearman rank correlation) were performed by using SPSS software. Statistical comparison between the groups (definitive infection group, clinical sepsis group and control group) was performed with analysis of variance (ANOVA) test for Continuous data. Sensitivity and specificity, and 95% confidence interval (CI) values were calculated for IL-8, CRP and PLT. Receiver-operating characteristic (ROC) curves were used for the determination of thresholds for the sepsis group versus healthy neonate group. The relationship between CRP and IL-8 was determined with the Spearman rank correlation test. The correlation coefficient and its 95% CI are presented. A *P*-value of <0.05 was considered statistically significant.

Findings

Of the 93 neonates recruited into the study, complete data were obtained for eighty consisting of 38 cases and 42 controls. Clinical and laboratory characteristics are shown in tables 1 and 2. No significant differences ($P>0.05$) were observed between the controls and cases with respect to weight, gestational age, Apgar score at fifth minute, sex, mode of delivery, maternal age, parity, history of pregnancy and delivery problem (Table 1).

The cases comprised infants who fulfilled the clinical criteria for sepsis ($n=20$) and those with a definitive diagnosis of infection ($n=18$). Among definitive infection subgroup, fifteen were blood-culture positive: six for gram negative bacilli, four for *Klebsiella pneumoniae*, three for *Escherichia coli*, and two for *Staphylococcus epidermidis*. CSF culture was positive for *Escherichia coli* in two and for gram negative bacilli in one patient.

Clinical features included lethargy ($n=28$), poor feeding ($n=23$), respiratory distress ($n=21$), apnea ($n=11$), seizures ($n=14$), fever ($n=10$), use of ventilation ($n=10$), abdominal distension ($n=9$), cyanosis ($n=3$), hypotension ($n=2$), irritability ($n=2$). Significant differences were observed between control and case groups regarding platelet count, while WBC, ANC, CRP and IL-8 ($P<0.05$, Table 2). IL-8 level was decreased in order of definitive infection, clinical sepsis and healthy subjects ($P<0.001$, Table 2). Of the 38 infants assigned to the disease group, 7 patients expired. Mean serum level IL-8 in non-survivors was 522.342 (316-727, 95% CI for mean) higher than in survivors (158.182, 101-215 95% CI, $P<0.001$). Figures 1 and 2 show that there was a sensitivity of 94.8%, specificity of 100%, positive predictive value (PPV) of 97.4%,

Table 1: Clinical characteristics of participant groups

	Definitive infection	Clinical sepsis	Healthy neonates	<i>P</i> -value
Number of participants	18	20	42	0.7
Gestational age (wk) mean (SD*)	34.5 (3.6)	36 (3.3)	36 (3)	0.5
Birth weight (g) mean (SD)	2140(630)	2320(740)	2210(690)	0.7

* SD: Standard Deviation

Table 2: Comparison of biochemical markers between groups

Marker (Unit)	Groups	Between groups	P-value		
			Infection and healthy infants*	Sepsis and healthy infants†	Sepsis and infection‡
IL-8 (pg/mL)	Definitive infection	510.86 (10.7-822)			
	Clinical sepsis	236.76 (11.2-728)	<0.001	<0.001	<0.05
	Healthy neonates	15.07 (0.4-50)			
CRP (mg/dl)	Definitive infection	22 (1-30)			
	Clinical sepsis	16.5 (2-28)	<0.001	<0.001	<0.01
	Healthy neonates	1 (0-13)			
Platelet ($\times 10^3$ cmm)	Definitive infection	196 (15-670)			
	Clinical sepsis	95 (12-370)	<0.05	<0.05	<0.01
	Healthy neonates	234 (28-648)			
WBC# (cmm)	Definitive infection	16300 (4100-29000)			
	Clinical sepsis	15800 (5000-35000)	<0.01	<0.01	>0.05
	Healthy neonates	9200 (4200-16000)			

* Definitive infection compared with healthy infants

† Clinical sepsis compared with healthy infants

‡ Clinical sepsis compared with definitive infection

White Blood Cells

and negative predictive value (NPV) of 100% at a cut-off value of 60 pg/ml for the IL-8 assay at presence of signs and symptoms. Also IL-8 concentration and the peak CRP concentration had sensitivities of 66%, Specificity of 100%, NPP of 82.7%, and PPV of 100% (Fig. 2). Thrombocytopenia was seen in 17 (44.7%) of patients and 8 (19%) of normal newborns.

Discussion

Serum IL-8 was significantly higher in infants with confirmed sepsis than healthy infants, prior to the blood culture being positive. Among the patients in confirmed sepsis group, not survived infants had significantly higher serum IL-8 levels than survived infants.

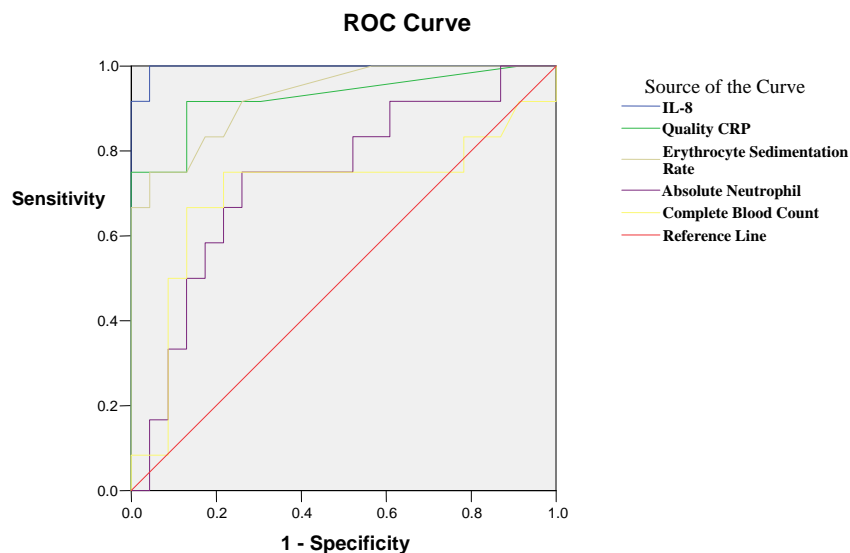
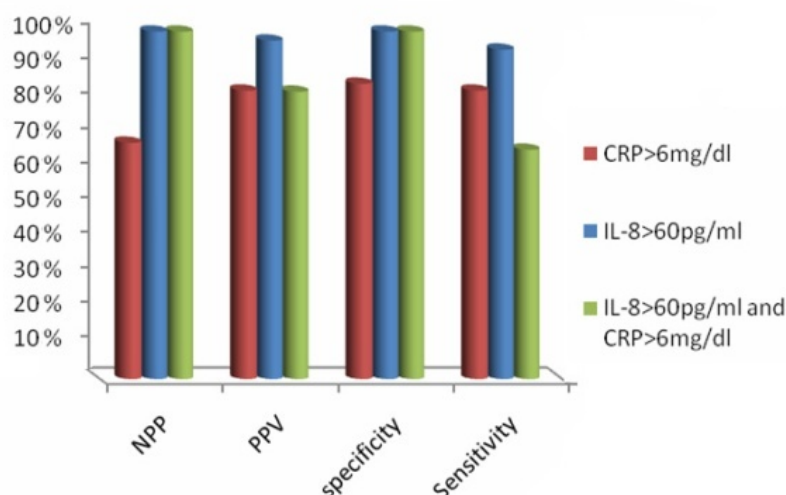


Fig. 1: Sensitivity and specificity of laboratory tests**Fig. 2:** Sensitivity, specificity, positive predictive value and negative predictive value of laboratory tests

The most common organism implicated in late onset sepsis were gram negatives (86.6%) and in 13% of cultures grew *Staphylococcus epidermidis*. In other studies, the most common organism implicated in late onset sepsis was *Staphylococcus epidermidis*^[9,13]. It is partially because too many patients attend NICU and the ratio of nurse to patient is low.

The role of IL-8 and CRP for early diagnosis of neonatal infection: Accurate and timely diagnosis of late onset neonatal sepsis remains challenging to the clinician and the biochemists.

The management of suspected neonatal sepsis would be greatly simplified if there were a single reliable marker of infection with results available soon after the onset signs and symptoms. A reliable prediction of the absence of infection would be most useful in the clinical setting as antibiotics could be withheld in more of these infants than is the current practice^[11,14]. The majority of suspected infective infants who receive antibiotics for 2–3 days because clinical signs suggest possible infection and early laboratory parameters are unable to rule out infection^[15]. Less than 10 percent of the neonates evaluated and treated for sepsis had definite infection^[16].

Cytokines are promising diagnostic markers and their levels are increased early in the infective process^[17]. We have found that serum

IL-8 concentrations are significantly higher in neonates who are subsequently proven by blood/CSF culture to have sepsis than for healthy infants. IL-8 concentrations in our infants with sepsis (510.86, range 27–822 pg/dl) were up to 34 times higher than in healthy neonates (15.07, range 0.4–50pg/dl). Consistent with our findings, other clinical studies have shown increased plasma levels of IL-8 in neonatal sepsis. Recently IL-8 was found to reduce in a cost-effective manner unnecessary antibiotic therapy for nosocomial bacterial infections in newborn infants by 73%^[1,18,19]. Serum IL-8 level has been reported to increase both in early- and late-onset neonatal sepsis and to have a sensitivity of about 80–91% and a specificity of about 76–100%^[20]. In a study by Edgar et al, who evaluated 60 infants with sepsis, all 25 infants with positive blood culture results had significant elevation of IL-6 and IL-8 levels^[9,21].

IL-8 is a cytokine that has a role in release, activation and chemotaxis of neutrophils^[2–4].

In the present study CRP ≥ 6 mg/dl as the cut-off value, for diagnosis definitive sepsis was a sensitivity of 83%, specificity of 85%, PPV of 83%, and NPV of 68%. In various studies using CRP ≥ 1 mg/dl as the cut-off value, the range of reported statistical outcomes is as follows; Sensitivity 70% to 93%, specificity 41% to 98%,

positive predictive accuracy 6% to 83%, and negative predictive accuracy 97% to 99%^[20,22]. In a study by Kocabas et al, in CRP ≥ 10 mg/dl sensitivity was 80.8%, specificity 100%, PPV 100%, and NPV 85.2%^[20]. CRP is a very good marker for sepsis after 12 h duration.

Although acute phase proteins have higher sensitivity and specificity, 10–30% of genuinely infected cases could still remain undiagnosed during the first 2 days of clinical presentation despite serial measurements.

IL-8 superiority to CRP in the detection of systemic inflammation is shown by the results in the present study. Here, in this study, CRP level correlated positively with IL-8 concentration, and PPV and specificity of the 2 assays were comparable. Franz et al studied IL-8 in combination with CRP and procalcitonin levels in 46 infants; 9 had culture positive bacterial infection. In their study, the combination of IL-8 (≥ 70 pg/ml) and/or CRP (> 10 mg/dl) was a reliable marker for the early diagnosis of bacterial infection in newborn infants. This had a sensitivity of 91% and a specificity of 73%^[9,18].

Association between serum level of IL-8 and severity of neonatal infection: In the present study, serum concentrations of IL-8 in non surviving neonates were 3.3 times higher than in surviving neonates (522.34 vs 155.18 pg/dl). Among neonates with confirmed sepsis, a non survivor infant had significantly high serum IL-8, which was also a predictor of mortality. The findings suggest that IL-8 level is related to severity of systemic inflammation. In adults with sepsis, serum levels of IL-8 are higher in nonsurvivors than in survivors^[1]. In the study of Franz et al^[1,18], a very premature infant who succumbed to a blood culture-proven sepsis had extremely high levels of circulating IL-8, greater than 10000pg/ml. Chemokines and proinflammatory cytokines are essential for host defense against microbial infection^[23], but excessive influx of activated leukocytes coupled with exaggerated production of potent proinflammatory mediators can contribute to deleterious consequences, leading to widespread small-vessel damage, multiorgan dysfunction, and death^[24].

One of our limitations of the present study was small sample size in subgroups of patients.

Conclusion

Serum level of IL-8 is a mediator of inflammation and can be used in diagnosis and prognosis of neonatal sepsis.

Serum IL-8 level was significantly higher in infants with confirmed infection than healthy newborns, prior to blood culture being positive and also it is associated with severity of infection. Further studies with large sample size in subgroups of suspected infective infants are needed to confirm our finding.

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Conflict of Interest: None declared.

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